ORIGINAL ARTICLE

Evaluation of the MB/BacT System for recovery of mycobacteria from clinical specimens in comparison to Lowenstein–Jensen medium

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Objective To evaluate the performance of the MB/BacT system (Organon Teknika) in comparison to Lowenstein–Jensen (LJ) solid medium for recovery of mycobacteria from clinical specimens.

Methods Two thousand three hundred and ten specimens (1626 respiratory, 593 urine, 60 body fluids, five tissue and 26 others) were inoculated in MB/BacT (0.5 mL) and on two LJ slants (0.25 mL each). *N*-acetyl-L-cysteine-NaOH (final concentration 2%) was used for decontamination.

Results Two hundred and fifty-one (10.8%) mycobacterial isolates [190 *Mycobacterium tuberculosis* complex (MTBC) and 61 non-tuberculous mycobacteria (NTM)] were detected. Of these 251 isolates, 234 (93.2%; 181 MTBC and 53 NTM) were detected in MB/BacT and 169 (67.3%; 154 MTBC and 15 NTM) on LJ. The mean (median) times to detection of MTBC by MB/BacT and LJ were 13.8 (13) and 22.1 (20) days, respectively, while overall contamination rates were 7.7% and 8.1%, respectively.

Conclusions Sensitivity and time to detection were significantly better with MB/BacT than with solid LJ medium.

Keywords *Mycobacterium*, MB/ BacT system, LJ medium, culture

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INTRODUCTION

Tuberculosis is on the increase throughout the world and remains one of the few infectious diseases diagnosis which often relies on clinical suspicion. The majority of routine clinical laboratories still depend on the acid-fast bacilli (AFB) smear and culture on solid media, usually Lowenstein–Jensen (LJ) diagnosis. Microscopy, although quick and easy, has poor sensitivity. Culture on solid media is more specific and sensitive, but can take several weeks. Liquid culture using a radiometric method (BACTEC 460 TB) is faster but cannot be implemented in every laboratory, for safety reasons. So far, the Standard has been the combination of the BACTEC 460 TB system and solid culture media [1]. Sensitivity, specificity and the low detection time of the radioactive method have been demonstrated in early studies [2,3]. More recently, new liquid media-based systems have been introduced for expedited isolation of the *Mycobacterium tuberculosis* complex (MTBC), for indirect susceptibility testing, and for shortened detection time in conjunction with nucleic acid probes [1,4,5]. These systems include: Becton-Dickinson's MGIT (Becton Dickinson, Cockeysville, MD, USA) and fluorescent 9000 MB system, Difco's ESP (Difco Laboratories, Detroit, MC, USA), and Organon Teknika's MB/BacT (Organon Teknika, Durham, NC, USA). The recent development of DNA amplification by the polymerase chain reaction (PCR) permits rapid detection of mycobacteria in clinical specimens. However, in practice, limitations of the method include problems of contamination, inhibition, and differences in sensitivity and specificity related to the

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particular target sequence for DNA amplification as well as the protocol employed [6,7].

Today, 'the driving force for the clinical laboratory is to provide ever more rapid species identification and antimycobacterial drug susceptibility profiles', because of altered clinical and epidemiological situations over recent years [1,8]. The emergence of clinically significant non-tuberculous mycobacteria (NTM), especially in immunocompromised hosts, poses an additional challenge for patient care.

The aim of this study was to evaluate the performance of the MB/BacT system (Organon Teknika) in comparison with culture on LJ medium.

MATERIALS AND METHODS

Specimens

Two thousand three hundred and ten specimens from 1138 patients, collected by the Clinical Microbiology Department of the Military Medical Academy, Belgrade, Yugoslavia between October 1998 and November 1999, were included in the study. Specimens processed for mycobacterial culture included 1626 respiratory specimens (sputum, bronchial washing and aspirate, pleural fluid, and pharyngeal swab), 593 urine samples, 60 normally sterile body fluids (pericardial fluid, cerebrospinal fluid, synovial fluid, and ascites), five tissue samples, and 26 others (stools, pus).

Specimen processing

Respiratory specimens, except pleural fluid, and contaminated site specimens were decontaminated by the addition of an equal volume of *N*-acetyl-L-cysteine-NaOH (final concentration 2%). After 15 min at room temperature this mixture was neutralized with phosphate-buffered saline (PBS) at pH 6.8 and concentrated by centrifugation at 3000 *g* for 15 min [1]. Specimens collected from sterile sites were concentrated by centrifugation and were cultured without prior decontamination. Tissue specimens were ground in sterile 0.9% NaCl before centrifugation. The sediment obtained after centrifugation was resuspended in 2 mL of PBS and was used for inoculation of the media.

Cultivation

Each MB/BacT bottle was inoculated with 0.5 mL of sediment, while two LJ slants were each inoculated with 0.25 mL of sediment. For the ligase chain reaction (LCR) probe (performed only for smear-positive specimens) 0.5 mL of sediment was used, and a small amount (except from urine specimens) was used to prepare for auramine staining [1]. MB/BacT bottles were loaded in the instrument for continuous monitoring over 42 days, LJ slants were incubated for 70 days at 37 °C (first 2 weeks in 5% CO_2) and were read for growth twice a week for the first 4 weeks and weekly thereafter. When growth was observed on LJ medium or signalled by MB/BacT instrument, an AFB smear was made to determine whether the cultures contained mycobacteria or contaminants. Samples of broth from MB/BacT bottles with a positive signal were subcultured on LJ slants. We were not looking for mycobacteria requiring other than 37 °C incubation temperatures, special growth factors, or prolonged incubation time.

Identification of mycobacteria

Mycobacterial isolates were identified using conventional methods [1]. For the LCR, theLCx *Mycobacterium tuberculosis* Assay was performed in accordance with the instructions of manufacturer (Abbott Diagnostics, Abbot Park, IL, USA). The amplification product was detected automatically by Microparticle Enzyme Immunoassay.

Statistical analysis

The statistical significance of differences in recovery rates was determined by the χ^2 test (number of culture-positive specimens) and proportion *t*-test (number of patients with tuberculosis). The statistical differences in isolation times (only the paired values used) were determined by the Wilcoxon signed-rank test. Clinical assessment included by review a tuberculosis expert as well as consideration of the results obtained from additional specimens.

RESULTS

Specimens and patients

Mycobacteria were isolated from 251 (10.9%) of the total of 2310 clinical specimens. From 593 specimens of urine (smear examinations were not performed), we obtained 18 isolates of mycobacteria (15 MTBC and three NTM); and from the total of the other 1717 specimens, for which smear exam-

Table 1 Rates of recovery of MTBC and NTM isolates by MB/BacT and LJ culture systems from 2310 specimens

Species (no. of isolates)	Number (%) of isolates by MB/BacT system	Number (%) of isolates by LJ system	*P*-value
MTBC (190)	181 (95.3)	154 (81.1)	<0.001
NTM (61)	53 (86.9)	15 (24.6)	<0.001
Total (251)	234 (93.2)	169 (67.3)	<0.001

MTBC, *Mycobacterium tuberculosis* complex; NTM, non-tuberculous mycobacteria; LJ, Lowenstein–Jensen.

Table 2 Smear and culture results for 175 MTBC-positive cultures

Smear result[a]	No. of smears	Number of positive cultures (% recovery) MB/BacT	Number of positive cultures (% recovery) LJ	*P*-value
Positive	125	120 (96)	99 (79.2)	<0.001
Negative	50	46 (92)	42 (84)	NS
Total	175	166 (94.9)	141 (80.6)	<0.001

MTBC, *Mycobacterium tuberculosis* complex; LJ, Lowenstein–Jensen; NS, not significant.
[a]Smear examinations were performed for 1717 specimens.

inations were performed, 233 (13.6%) isolates of mycobacteria were recovered (175 MTBC and 58 NTM): 125 (53.6%) from acid-fast smear-positive and 108 (46.4%) from acid-fast smear-negative specimens.

NTM were detected in 61 specimens from 52 patients. None of the specimens that were culture-positive for NTM were smear-positive. NTM were recovered from sputa (55 isolates from 46 patients), bronchial washings (three isolates from three patients), and urines (three isolates from three patients). None of these patients had clinical evidence of mycobacterial disease. Since all isolates of NTM were considered as contaminants, and the patients were not treated with antimycobacterial agents, these isolates were neither further identified nor included in detailed evaluations.

Most of the isolates of MTBC (173) were recovered from respiratory specimens, 15 from urines, one from pericardial fluid, and one from lymph node. In total 69 patients were detected, from whom MTBC were recovered for the first time, and three patients in whom it was recovered following response to treatment. In five patients MTBC were recovered in urines by both the MB/BacT and LJ culture systems (smear examinations were not performed). From the other specimens MTBC were isolated from 67 patients: 65 patients from respiratory specimens (included two from pleural fluids and three following a response to treatment), one from pericardial fluid, and one from lymph node biopsy.

Recovery rates by MB/BacT system and LJ

The overall recovery rate of mycobacteria by the MB/BacT system was higher than that with LJ (Table 1). The smear and culture results for 175 MTBC culture-positive specimens are shown in Table 2. MTBC grew from 125 (80.6%) of 155 smear-positive specimens. The 30 smear-positive culture-negative specimens (Table 3) included 21 specimens from three patients with previously positive cultures of MTBC during treatment, eight specimens from patients with previously positive

Table 3 Culture results of smear-positive, culture-negative specimens[a]

Culture in MB/BacT and on LJ slants	No. of specimens
Both contamination	10
LJ contamination; MB/BacT-negative	5
MB/BacT contamination; LJ-negative	3
Both negative	12
Total	30

LJ, Lowenstein–Jensen.
[a]Smear examinations were performed for 1717 specimens.

cultures of MTBC in less than 7 days, and one pharyngeal swab from a patient with documented pulmonary tuberculosis and a positive MTBC LCR probe.

Of the 175 culture-positive specimens that grew MTBC, the MB/BacT and LJ results were culture negative on nine and 34 occasions, respectively. Six of the nine isolates missed by the MB/BacT system were lost bacause of contamination; of the 34 isolates missed by LJ, 24 were lost because of contamination. Thus, the MB/BacT system and LJ slants offered an advantage for the growth *per se* in 10 and three cases, respectively.

There were three patients in whom we detected MTBC by positive smear results (cultures in MB/BacT and on LJ were contaminated or negative), confirmed by LCR probes: one patient with pulmonary tuberculosis from whom we received only one specimen as a pharyngeal swab and two patients with pulmonary tuberculosis following a response to treatment (Table 4). The MB/BacT system revealed six patients in whom LJ cultures were negative or contaminated: four patients with pulmonary tuberculosis, one patient with tuberculous pleurisy, and one patient with pulmonary tuberculosis during follow-up of treatment. LJ cultures revealed three patients in whom MB/BacT cultures were negative or contaminated: two patients with pulmonary tuberculosis and one patient with lymph node tuberculosis. There was no statistical significance in differences of recovery rates between MB/BacT and LJ culture system.

Table 4 Rates for patients in whom we detected MTBC by different methods from 1717 specimens[a]

Method	No. (%) of patients
AFB smear-positive, culture-negative; confirmed by LCR probes	3 (4.5)[b]
MB/BacT only	6 (9.0)[c]
LJ only	3 (4.5)
MB/BacT total	61 (91.0)[d]
LJ total	58 (86.6)[d]
Total	67 (100.0)

MTBC, *Mycobacterium tuberculosis* complex; AFB, acid-fast bacilli; LCR, ligase chain reaction; LJ, Lowenstein–Jensen
[a]The staining was not done for urine specimens; from 593 urine specimens we isolated MTBC in five patients by both MB/BacT and LJ media, simultaneously.
[b]Included two patients following a response to treatment.
[c]Included one patient following a response to treatment.
[d]Not significant.

Contamination rate

The contamination rate for all specimens inoculated in the MB/BacT system was 7.7% (177 specimens of 2310). A total of 8.1% (186 of 2310) of both LJ slants were contaminated. The contamination rate of specimens in which MTBC were detected (205) was higher for LJ (19%) than for the MB/BacT system (9.3%).

Mean (median) time to detection

The overall mean (median) times to detection of MTBC were 14.2 (13) days for the MB/BacT system and 22.4 (20) days for LJ ($P < 0.001$). The mean (median) time to detection of NTM by MB/BacT was 19.3 (23) days. Times to detection of NTM by LJ were not recorded. The mean and median times required for the recovery of MTBC according to the smear results by both culture systems is presented in Table 5.

The mean (median) times for detection of the patients with tuberculosis, taking into account the first three specimens, for the MB/BacT and LJ culture system were 13.7 (12) and 21.7 (18) days, respectively ($P < 0.001$). For the patients for whom all specimens were smear-negative the mean (median) times to detection by MB/BacT and LJ were 20.6 (17.5) and 33.3 (27) days, respectively ($P < 0.01$).

Table 5 Paired mean (range) and median time (in days) to detection of MTBC by MB/BacT system and LJ media according to the smear results

Smear result[a]	Mean (range) and median time		*P*-value
	MB/BacT system	LJ slants	
Positive	11.3 (4–36) 10	18.1 (10–41) 18	<0.001
Negative	19.6 (5–40) 18	31.2 (15–71) 28	<0.001
Total	13.8 (4–40) 13	22.1 (10–71) 20	<0.001

MTBC, *Mycobacterium tuberculosis* complex; LJ, Lowenstein–Jensen.
[a]Staining was not performed for 593 urines specimens.

DISCUSSION

For the detection of mycobacteria, liquid-based media such as BACTEC 460 and the Mycobacteria Growth Indicator Tube (MGIT) have generally been found to be more sensitive and to require fewer days to become positive than do conventional solid media [4,5]. The MB/BacT, like BACTEC 460 and MGIT, processes growth by detection of metabolic changes that occur in the medium. In the literature, there are few clinical studies of the MB/BacT system. The study design used in this investigation was somewhat biased in favor of the LJ culture system in that LJ slopes were incubated for 10 weeks while MB/BacT bottles were incubated for 6 weeks. Under the conditions of this study, the MB/BacT system compared favourably with LJ slants in terms of sensitivity (93.2% vs. 67.3%; $P < 0.001$). Published rates of *Mycobacterium* spp. recovery range from 73.0% to 96.5% for the MB/BacT system [9–13] and 53.6% to 95.9% for the LJ culture system [10–13].

One of the disadvantages often mentioned about culture in liquid medium is that it does not provide visible colonies. This could increase the time required for identification and susceptibility testing of the isolate. However, the direct testing of positive MB/BacT broth by AccuProbe, without prior centrifugation, allows for the accurate and rapid identification of MTBC [9]. Additionally, the presence of cords in MB/BacT broth is a reliable criterion for rapid, predictive identification of MTBC for laboratories with a high proportion of MTBC when the smears are examined by a microbiologist who has experience with AFB staining [9].

The recovery rate for NTM isolates on LJ was lower than that by the MB/BacT system, 24.6% and 86.9%, respectively. It is well-known that LJ is not the best medium for the isolation of NTM isolates, because LJ is designed primarily for the growth of MTBC [13]. No patient from whom NTM were isolated showed clinical evidence of mycobacterial disease. Thus, one could argue that detecting these isolates increases the apparent contamination rate of MB/BacT bottles by an additional 2.6%. However, approximately 2–10% of all mycobacterial infections in the USA are due to NTM, and a higher incidence may be observed in patients with malignancy and acquired immunodeficiency syndrome [8].

The rates of recovery of MTBC in this study using the MB/BacT system and the LJ culture system were 95.3% and 81.1%, respectively ($P < 0.001$). Published rates of MTBC recovery range from 80.2% to 98.7% [11,13] for the MB/BacT system and 67.6% to 95.9% for the LJ system [10,13].

From the infection control standpoint, the speed of MTBC detection is most critical. In this study, the overall mean times (urines included) to detect MTBC were 14.2 and 22.4 days for MB/BacT and LJ slants, respectively ($P < 0.001$). Others [12,14,15] have shown that the MB/BacT system requires much less time to detect MTBC than do LJ slants (13.7–14.2 vs. 26.1–29.4 days).

In this study, the mean times to detection of MTBC from smear-positive specimens for the MB/BacT and LJ were 11.3 and 18.1 days, respectively ($P < 0.001$). Published mean times to detection of MTBC for smear-positive specimens for the MB/BacT and LJ cultures were 11.5 vs. 16.7 days [16]. For the smear-negative specimens the mean times to detection of MTBC for the MB/BacT and LJ were 19.6 and 31.2 days, respectively ($P < 0.001$). Published mean times to detection of MTBC for smear-negative specimens range from 19.3 to 21 days for the MB/BacT system [13,16]and 26.3 for LJ slants [17].

Published contamination rates range from 1.8% to 8.4% for the MB/BacT system [17,18] and 1.5% to 13.3% for the LJ system [12,17]. In our study, overall contamination rates were 7.7% and 8.1% for MB/BacT and LJ, respectively. Surprisingly, the culture failures of LJ medium were primarily from smear-positive specimens. The contamination rates in smear-positive specimens for the MB/BacT system and LJ medium were 11% and 23.9%, respectively, while in smear-negative specimens the contamination rates were much lower for both of them (4% each).

In this study there were 30 smear-positive, culture-negative specimens, mainly from three patients (21 specimens) during treatment. All 12 smear-positive, culture-negative (MB/BacT and LJ) specimens (Table 3) derived from patients during treatment. Unfortunately, the incubation times of MB/BacT bottles for these specimens were not prolonged.

In conclusion, the sensitivity and time to detection of MTBC for the MB/BacT system were comparable to those obtained in published works [10–13,15,16] while the contamination rate was slightly higher [10–13,15,18]. Neither the MB/BacT nor the LJ culture system detected all of the MTBC-positive specimens and patients with tuberculosis. The

present work indicates that the MB/BacT system has great potential: it is a rapid, sensitive, efficient, safe, simple-to-use, and fully automated method for the isolation of mycobacteria.

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